Plasma Homocysteine Level and Coronary Artery Disease in Type 2 Diabetic Patients Without Nephropathy

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Abstract

Background- Elevated plasma homocysteine (Hcy) is considered to be a novel risk factor for coronary atherosclerosis. Considering the important role of the kidneys in Hcy clearance, the relation, if any, between Hcy and diabetes mellitus (DM) and coronary artery disease (CAD) in diabetic patients is still unclear. The aim of this study was to investigate whether plasma Hcy is a predictor of CAD in patients with type 2 DM without any evidence of nephropathy.

Methods- Among type 2 DM patients without nephropathy (cr ≤1.2 mg/dl) referring for coronary angiography to Tehran Heart Center during 2005, 151 patients were evaluated in this cross-sectional study. CAD was confirmed if there was a lumen diameter narrowing >50 percent in at least one coronary artery. The extent of CAD was determined by the number of affected coronary arteries.

Results- The mean±SD Hcy level was 11.35±3.7 μmol/l. No significant difference was observed between the CAD and control groups (11.52±4.01 μmol/l vs. 11.02±3.11 μmol/l, p=0.440). CAD was related to body mass index (p=0.044), systolic blood pressure (p=0.027), HDL-c level (p=0.016), serum creatinine (p=0.042), and HbA1c level (p=0.001). A binary logistic regression analysis found systolic blood pressure (OR: 0.96, p=0.003), creatinine (OR: 24.76, p=0.013), and HbA1c (OR: 2.41, p=0.017) as independent predictors of the presence of CAD. Predictors of the extent of CAD were history of hypertension and current smoking.

Conclusion- In the presence of normal renal function, plasma Hcy level cannot predict either presence or extent of CAD in patients with type 2 DM without nephropathy***(Iranian Heart Journal 2009; 10 (4):6 -13).***

Key words: coronary artery disease ■ homocysteine ■ diabetes mellitus

Coronary artery disease (CAD) is the leading cause of morbidity in patients with type 2 diabetes mellitus (DM), and the prevalence of atherosclerosis is 2-6 fold greater in diabetic patients than in their non-diabetic counterparts.[1]

An extensive number of clinical and experimental studies have demonstrated that a mild increase in plasma homocysteine (Hcy) level may be an independent risk factor for cardiovascular diseases.[2-4]

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A meta-analysis of 27 observational studies has reported that up to 10% of CAD could be explained by hyperhomocysteinemia.[5] However, the exact mechanism by which Hcy causes atherosclerosis has not been clearly explained.

Previously published studies have reported different and even contradictory plasma Hcy levels in diabetic populations.[1, 6-14]

This controversy may be related to the heterogeneity of the populations studied; particularly patient renal function. One possible explanation for different levels of plasma Hcy may be the important role of the kidneys in Hcy metabolism. In the early stages of diabetic nephropathy, plasma Hcy concentration is usually low or normal. This may be due to glomerular hyperfiltration. However, once diabetic nephropathy is established, a reduced glomerular filtration rate results in higher levels of plasma Hcy.[15-17]

It is not clearly known whether plasma Hcy level is independently associated with CAD in patients with type 2 DM or whether it differs as a consequence of altered renal function.

Therefore, the aim of this study was to investigate whether plasma homocysteine level is an independent predictor of presence or extent of CAD in patients with type 2 DM without nephropathy.

Methods

Patients

The study population of this cross-sectional study consisted of 151 patients (66 men, 85 women) with type 2 DM who underwent coronary angiography in Tehran Heart Center (a referral hospital affiliated to Tehran University of Medical Sciences) between April and October 2005.

DM was defined if patients were under active treatment with insulin or oral hypoglycemic agents. For patients on dietary treatment, diabetes was defined based on the criteria from the American Diabetes Association (ADA) for the diagnosis and classification of diabetes.[18] Patients with acute myocardial infarction, hypothyroidism, or renal failure (creatinine ≥1.2 mg/dL) were not enrolled in this study.

Coronary angiography was performed by experienced interventional cardiologists, who were blinded to the study protocol. CAD was confirmed if there was a lumen diameter narrowing more than 50 percent at least in one coronary artery. The extent of CAD was determined according to the standard method into single-, two-, or three-vessel disease. Minimal CAD was defined as a <50% narrowing in lumen diameter.

Based on the angiographic results, the patients were divided into two groups: the CAD group consisted of 100 patients with CAD or minimal CAD, and the control group comprised 51 patients without CAD.

All the participants gave written consent for the angiographic examination. The study protocol was approved by the Ethics Committee of Iran University of Medical Sciences and investigations were conducted in accordance with the Declaration of Helsinki.

Clinical assessments

The patients underwent a complete clinical examination, and a comprehensive medical history was recorded. Blood pressure, body mass index (BMI), known cardiovascular risk factors, family history of CAD, history of hypertension or hyperlipidemia, and duration of diabetes were also identified.

Biochemical measurements

Blood samples were taken after an overnight fast and fasting blood sugar (FBS), total cholesterol (TC), triglycerides (TG), low-density lipoprotein (LDL-c), high-density lipoprotein (HDL-c), lipoprotein a [Lp(a)], serum creatinine (Cr), and glycosylated hemoglobin (HbA1c) were measured. Plasma Hcy was determined by high-performance liquid chromatography (HPLC). All th emeasurements were performed in the laboratory of Tehran Heart Center with the same methods for all the blood samples, and

the laboratory personnel were unaware of the study protocol and clinical / angiographic data.

Statistics

All the statistical analyses were performed using SPSS for Windows 15.0 (SPSS Inc., Chicago, Illinois). The data are presented as frequency (percentages) for the categorical variables and mean ± SD for the continuous variables.

Differences between the groups were assessed using the Chi-square, independent samples t test, and one-way ANOVA. Variables found to be predictors of CAD in the univariate analysis were then entered into a multivariate model using the binary logistic regression to determine the independent predictors of CAD. The Hosmer and Lemeshow tests for Goodness-of-Fit were used. A 2-tailed *P*-value of <0.05 was considered statistically significant.

Results

Baseline characteristics of study population

The median age of the study population was 49 years (ranging from 29 to 55), and their median duration of diabetes was 3 years.

According to the results of coronary angiography, of 100 patients with CAD, 15 had minimal CAD, 33 had single-vessel, 23 had two-vessel, and 29 patients had three-vessel disease. The demographic and clinical features of these patients are presented in Table I.

Laboratory values for the CAD patients and their control counterparts are listed in Table II.

Male gender was significantly more common in the CAD group than in the control group (54% vs. 23.5%, $p<0.001$). Clinical and biochemical measurements were similar in the two groups except for reduced systolic blood pressure and serum HDL-c level as well as higher HbA1c in the CAD group ($p<0.05$). Compared to the control group, creatinine was significantly higher in the CAD patients; however, both were within the normal range.

Table I. Demographic features of type 2 diabetic patients in CAD and control groups.

Characteristics	CAD patients (n=100)	Controls (n=51)	*P*- value
Age (*year*)	47.88 ±5.20	48.66±5.63	.395
Gender (Men)	54 (54)	12 (23.5)	**<.001***
BMI (kg/m^2)	28.62±5.21	30.48±5.29	**.044***
Current smoking	20 (20)	4 (7.8)	.053
Positive family history of CAD	32 (32)	15 (29.4)	.745
Hyperlipidemia	74 (74)	38 (74.5)	.946
Hyperlipidemia duration (*month*)	53.37±63.54	50.38±48.87	.814
Hypertension	54 (54.5)	29 (56.9)	.787
Hypertension duration (*month*)	56.75±62.6	60.93±74.34	.793
Systolic blood pressure (*mmHg*)	124.25±19.47	132.72±21.30	**.027***
Diastolic blood pressure (*mmHg*)	80.38±10.80	82.32±9.65	.327
Diabetes duration (*month*)	66.18±70.23	68.44±67.40	.858
Insulin use	13 (13.1)	4 (8)	.106

*Significant group differences were detected ($p<0.05$)

Table II. Biochemical measurements for type 2 diabetic patients in CAD and control groups

Characteristics	CAD patients (n=100)	Controls (n=51)	*P* -value
Total cholesterol (*mg/dL*)	203.97±56.65	211.88±119.29	.586
HDL-c (*mg/dL*)	40.75±8.66	45.00±12.36	**.016***
LDL-c (*mg/dL*)	114.05±34.94	109.40±34.34	.474
Triglyceride (*mg/dL*)	301.90±482.32	250.29±176.63	.462
Fasting blood sugar (*mg/dL*)	160.48±61.59	164.67±59.59	.695
Creatinine (*mg/dL*)	0.99±0.21	0.92±0.20	**.042***
Lipoprotein (a) (*mg/dL*)	40.27±34.00	31.05±28.57	.133
Total plasma homocysteine (*µmol/dL*)	11.52±4.01	11.02±3.11	.442
Hb A_1C (%)	7.32±0.75	6.90±0.71	**.010***

*Significant group differences were detected ($p<0.05$)

Considering the extent of CAD, only the patients' history of hypertension and current smoking was found to have a statistically significant difference among the categories (p<.05, Table III).

Plasma homocysteine level

Plasma Hcy level ranged from 1.10 to 35.00 μmol/L, and the mean ± SD Hcy level was 11.35± 3.7 μmol/L. No significant difference was observed between the CAD and control groups (11.52 ± 4.01 μmol/L vs. 11.02 ± 3.11 μmol/L, 95% Confidence Interval [CI] of difference = -.77-1.76, p =0.440). Although the mean plasma Hcy level was highest in The patients with minimal CAD (13.23±6.6 μmol/L) and lowest in patients with two-vessel disease (10.76 ± 2.9 μmol/L), there was no statistically significant difference among the four categories of CAD extent as depicted in Table IV.

Table III. Demographic features of type 2 diabetic patients with CAD according to the extent of CAD

Characteristics	Minimal CAD (n=15)	1-vessel disease (n=33)	2-vessel disease (n=23)	3-vessel disease (n=29)	*P*- value
Age (*year*)	45.67±6.6	48.51±4.1	48.39±5.9	47.90±4.8	.335
Men	5 (33.3)	16 (48.5)	13 (56.5)	20 (69)	.130
BMI (kg/m^2)	30.23±5.0	28.03±4.6	29.04±6.2	28.15±5.2	.536
Current smoking	2 (13.3)	5 (21.7)	6 (26.1)	7 (24.1)	**.041***
Positive family history of CAD	3 (20)	11 (33.3)	6 (26.1)	12 (41.4)	.448
Hyperlipidemia	11 (73.3)	25 (75.7)	18 (78.3)	20 (69)	.884
Hyperlipidemia duration (*month*)	60.45±65.7	63.17±69.3	48.07±64.7	39.65±53.6	.668
Hypertension	12 (80)	21 (63.6)	10 (43.5)	11(38)	**.032***
Hypertension duration (*month*)	46.75±53.4	61.09±58.4	58.22±73.4	59.25±82.1	.940
Systolic blood pressure (*mmHg*)	129.61±20.4	121.73±22.7	118.12±13.9	128.00±17.8	.272
Diastolic blood pressure (*mmHg*)	82.31±11.6	79.65±10.7	75.96±6.1	83.00±12.2	.196
Diabetes duration (*month*)	77.33±102.2	65.43±60.8	66.75±65.9	59.96±64.9	.904
Insulin use	3 (20)	1 (3.3)	5 (21.7)	4 (13.8)	.153

*Significant group differences were detected (p <0.05)

Table IV. Biochemical measurements for type 2 diabetic patients with CAD according to the extent of CAD

Characteristics	Minimal CAD (n=15)	1-vessel disease (n=33)	2-vessel disease (n=23)	3-vessel disease (n=29)	*P* - value
Total cholesterol (*mg/dL*)	203.33±42.6	195.88±38.1	201.67±33.6	216.00±88.6	.594
HDL-c (*mg/dL*)	43.33±8.6	41.14±9.0	40.62±7.7	38.93±8.9	.463
LDL-c (*mg/dL*)	108.31±28.6	110.08±38.4	115.94±32.6	119.72±36.8	.717
Triglyceride (*mg/dL*)	392.80±593.1	250.82±136.2	241.95±124.6	360.44±781.8	.660
Fasting blood sugar (*mg/dL*)	143.67±56.0	160.36±64.7	167.13±62.7	164.3±61.4	.691
Creatinine (*mg/dL*)	0.97±0.3	0.95±0.2	0.98±0.2	1.08±0.2	.116
Lipoprotein (a) (*mg/dL*)	25.15±21.8	48.78±30.7	48.38±50.4	31.33±19.2	.076
Total plasma homocysteine (*μmol/dL*)	13.23±6.6	11.70±4.0	10.76±2.9	11.05±2.7	.265
Hb A1C (%)	7.14±1.0	7.30±0.5	7.20±0.7	7.58±0.8	.367

Predictors of the presence of CAD

A binary logistic regression analysis with the presence of CAD as the dependent variable and all significant correlates of CAD as independent variables found systolic blood pressure (OR: 0.96, *p*=0.003), creatinine (OR: 24.76, *p*=0.013), and HbA1c (OR: 2.41, *p*=0.017) as independent predictors of the presence of CAD in patients with DM (Table V). The Hosmer and Lemeshow Goodness-of-Fit was not significant (Chi-square:10.301, d*f*: 8, *p*=0.245)

Table V. Independent predictors of the presence of CAD in type 2 diabetic patients without nephropathy

Factors	Beta	SE	*P* value	OR (95%CI)
Systolic blood pressure	-.041	.014	.003	.960 (.934-.986)
Creatinine	3.209	1.286	.013	24.756 (1.990-307.918)
HbA1C	.878	.369	.017	2.407 (1.168-4.959)

Discussion

Recently, more attention has been paid to the Hcy level as a novel risk factor for developing atherosclerosis and consequently cardiovascular events.[19-21] The relation, if any, between plasma Hcy level and DM is still a matter of controversy and debate among investigators.[22,23] In the present study, we aimed to explore the relationship between CAD and plasma Hcy levels in patients with type 2 DM without any evidence of nephropathy.

Some previously published reports introduced Hcy as an independent predictor of CAD in diabetic patients.[24,25] This association was not observed in our study. We found that in diabetic patients, both with and without CAD, plasma Hcy level was within the normal range and did not differ significantly. This observation suggests that the relation observed in other studies may not be independent of other factors which can affect the plasma Hcy level or the presence of CAD.

One of the most important factors suggested to be related to the plasma Hcy level is renal function and consequently the ability of kidneys in the clearance of Hcy. Diabetic nephropathy is one of the main complications of DM and occurs frequently in these patients. We found that in the presence of normal renal function, Hcy cannot predict the presence of CAD in patients with type 2 DM. Although this finding is in contrast with some observations,[16,25] it is in agreement with some other data.[10,26] A study conducted by Ndrepepa et al. found impaired renal function as a strong predictor of Hcy level.[6] They found that diabetes is associated with increased plasma levels of Hcy only in patients with impaired renal function. Conversely, in patients with preserved renal function, diabetes was not associated with the increased plasma Hcy level.

In our study, only patients with normal renal function were assessed and patients with any evidence of impaired renal function were excluded. Although we found higher levels of serum creatinine in patients with CAD, it was within the normal range both in the CAD and control groups (Table II). Therefore, the equivalence of plasma Hcy level between these two groups shows that Hcy may not be an independent correlate of atherosclerosis.

The independent predictors of the presence of CAD in our patients were decreased systolic blood pressure and increased levels of serum creatinine and HbA1c. These findings suggest other risk factors to be more important in the pathogenesis of atherosclerosis rather than Hcy in diabetic populations. Other significant correlates of the presence of CAD in the univariate analysis (BMI and HDL-c) were no longer present in the multivariate model. The Hosmer-Lemeshow Goodness-of-Fit test demonstrated a good fit (Chi-square:10.301, d*f*: 8, *p*=0.245).

No relationship between plasma Hcy level and the extent of CAD assessed by the number of affected coronary arteries was seen, although Bozkurt et al.[27] found that elevated Hcy levels in patients with CAD

correlates with the angiographic extent of atherosclerotic disease. This could be explained by the different methods which they used for the assessment of the extent of CAD, which determines a quantitative measure of CAD for each patient. The only factors found to be predictors of the extent of CAD in our patients were the history of hypertension and current smoking (Table III).

The present study confronted some limitations. As indicated previously,[28-30] inter-individual variation in plasma Hcy levels is regulated by the interaction of genetic and nutritional factors, in which dietary habits such as the intake of vitamins (folic acid, vitamin B6 and B12) play an important role.[31] Also, C677T polymorphism in the methylene-tetrahydrofolate reductase (MTHFR) gene has been reported to be related to the plasma Hcy level.[32] We did not evaluate these possible affecting factors; these may, therefore, act as confounding variables which can alter the levels of plasma Hcy independent of other risk factors of CAD. In addition, the sample size in the control group was relatively small. This was due to the fact that among patients with type 2 DM referring to our center for coronary angiography, it is somewhat difficult to find patients with normal coronary arteries.

In conclusion, we found that in the presence of normal renal function, plasma homocysteine level cannot predict either the presence or the extent of CAD in patients with type 2 DM without any evidence of nephropathy.

Conflict of Interest

No conflicts of interest have been claimed by the authors.

References

1. Audelin MC, Genest JR. Homocysteine and cardiovascular disease in diabetes mellitus. Atherosclerosis 2001; 159 (2): 497-511.

2. Matteucci E, Rossi L, Mariani S, Fagnani F, Quilici S, Cinapri V, et al. Blood levels of total homocysteine in patients with type 1 diabetes (with no complications, diabetic nephropathy and/or retinopathy) and in their non-diabetic relatives. Nutr Metab Cardiovasc Dis 2002; 12 (4): 184-9.

3. Rudy A, Kowalska I, Straczkowski M, Kinalska I. Homocysteine concentrations and vascular complications in patients with type 2 diabetes. Diabetes Metab 2005; 31 (2): 112-7.

4. Vrentzos G, Papadakis JA, Malliaraki N, Zacharis EA, Katsogridakis K, Margioris AN, et al. Association of serum total homocysteine with the extent of ischemic heart disease in a Mediterranean cohort. Angiology 2004; 55 (5): 517-24.

5. Boushey CJ, Beresford SA, Omenn GS, Motoulsky AG. A quantitative assessment of plasma homocysteine as a risk factor for vascular disease. JAMA 1995; 274: 9.

6. Ndrepepa G, Kastrati A, Braun S, Koch W, Kölling K, Mehilli J et al. Circulating homocysteine levels in patients with type 2 diabetes mellitus. Nutr Metab Cardiovasc Dis 2008; 18 (1): 66-73.

7. Wiltshire E, Thomas DW, Baghurst P, Couper J. Reduced total plasma homocysteine in children and adolescents with type 1 diabetes. J Pediatr 2001; 138: 6.

8. Smulders YM, Rakic M, Slaats EH, Treskes M, Sijbrands EJ, Odekerken DA et al. Fasting and post-methionine homocysteine levels in NIDDM. Determinants and correlations with retinopathy, albuminuria, and cardiovascular disease. Diabetes Care 1999; 22 (1): 125-32.

9. Emoto M, Kanda H, Shoji T, Kawagishi T, Komatsu M, Mori K, et al. Impact of insulin resistance and nephropathy on homocysteine in type 2 diabetes. Diabetes Care 2001; 24 (3): 533-8.

10. Agullo-Ortuno MT, Albaladejo MD, Parra S, Rodríguez-Manotas M, Fenollar M, Ruíz-Espejo F, et al. Plasmatic homocysteine concentration and its relationship with complications associated to diabetes mellitus. Clin Chim Acta 2002; 326 (1-2): 105-12.

11. Araki A, Sako Y, Ito H. Plasma homocysteine concentrations in Japanese patients with non-insulin-dependent diabetes mellitus: effect of

parenteral methylcobalamin treatment. Atherosclerosis, 1993; 103 (2): 149-57.

12. Wollesen F, Brattström L, Refsum H, Ueland PM, Berglund L, Berne C. Plasma total homocysteine and cysteine in relation to glomerular filtration rate in diabetes mellitus. Kidney Int 1999; 55 (3): 1028-35.

13. Oishi K, Nagake Y, Yamasaki H, Fukuda S, Ichikawa H, Ota K, et al. The significance of serum homocysteine levels in diabetic patients on haemodialysis. Nephrol Dial Transplant 2000; 15 (6): 851-5.

14. Mazza A, Bossone E, Mazza F, Distante A. Reduced serum homocysteine levels in type 2 diabetes. Nutr Metab Cardiovasc Dis 2005; 15 (2): 118-24.

15. Wijekoon EP, Brosnan ME, Brosnan JT. Homocysteine metabolism in diabetes. Biochem Soc Trans 2007; 35 (Pt 5): 1175-9.

16. Akalin A, Alatas O, Colak O. Relation of plasma homocysteine levels to atherosclerotic vascular disease and inflammation markers in type 2 diabetic patients. Eur J Endocrinol 2008; 158 (1): 47-52.

17. Chico A, Pérez A, Córdoba A, Arcelús R, Carreras G, de Leiva A et al. Plasma homocysteine is related to albumin excretion rate in patients with diabetes mellitus: a new link between diabetic nephropathy and cardiovascular disease? Diabetologia 1998; 41 (6): 684-93.

18. Report of the expert committee on the diagnosis and classification of diabetes mellitus. Diabetes Care 2003; 26 Suppl 1: S5-20.

19. Shammas NW, Dippel EJ, Jerin M, Toth PP, Kapalis M, Reddy M, Harb H. Elevated levels of homocysteine predict cardiovascular death, nonfatal myocardial infarction, and symptomatic bypass graft disease at 2-year follow-up following coronary artery bypass surgery. Prev Cardiol 2008; 11 (2): 95-9.

20. Soinio M, Marniemi J, Laakso M, Lehto S, Rönnemaa T. Elevated plasma homocysteine level is an independent predictor of coronary heart disease events in patients with type 2 diabetes mellitus. Ann Intern Med 2004; 140 (2): 94-100.

21. Nygard O, Nordrehaug JE, Refsum H, Ueland PM, Farstad M, Vollset SE. Plasma homocysteine levels and mortality in patients with coronary artery disease. N Engl J Med 1997; 337 (4): 230-6.

22. Taylor AJ, Feuerstein I, Wong H, Barko W, Brazaitis M, O'Malley PG. Do conventional risk factors predict subclinical coronary artery disease? Results from the Prospective Army Coronary Calcium Project. Am Heart J 2001; 141 (3): 463-8.

23. Splaver A, Lamas GA, Hennekens CH. Homocysteine and cardiovascular disease: biological mechanisms, observational epidemiology, and the need for randomized trials. Am Heart J 2004; 148 (1): 34-40.

24. Homocysteine a strong risk factor in diabetics. Harv Heart Lett, 2000; 10 (10): 6.

25. Anan F, Masaki T, Umeno Y, Yonemochi H, Eshima N, Saikawa T et al. Correlations between homocysteine levels and atherosclerosis in Japanese type 2 diabetic patients. Metabolism 2007; 56 (10): 1390-5.

26. Deepa R, Velmurugan K, Saravanan G, Karkuzhali K, Dwarakanath V, Mohan V. Absence of association between serum homocysteine levels and coronary artery disease in south Indian males. Indian Heart J 2001; 53 (1): 44-7.

27. Bozkurt E, Keles S, Acikel M, Islek M, Ateşal S. Plasma homocysteine level and the angiographic extent of coronary artery disease. Angiology 2004; 55 (3): 265-70.

28. Kolling K, Ndrepepa G, Koch W, Braun S, Mehilli J, Schömig A et al. Methylenetetrahydrofolate reductase gene C677T and A1298C polymorphisms, plasma homocysteine, folate, and vitamin B12 levels and the extent of coronary artery disease. Am J Cardiol 2004; 93 (10): 1201-6.

29. Das UN. Nutritional factors in the prevention and treatment of cardiovascular diseases. Agro Food Industry Hi-Tech 2007; 18 (3): 40-44.

30. Weir DG, Scott JM. Homocysteine as a risk factor for cardiovascular and related disease: nutritional implications. Nutrition Research Reviews 1998; 11 (2): 311-338.

31. AG M. Nutritional ecogenetics: homocysteine related arteriosclerotic vascular disease, neural tube defects, and folic acid. Am J Hum Genet 1996; 58: 4.

32. Dedoussis GV, Panagiotakos DB, Chrysohoou C, Pitsavos C, Zampelas A, Choumerianou D et al. Effect of interaction between adherence to a Mediterranean diet and the methylenetetrahydrofolate reductase 677C-->T mutation on homocysteine concentrations in healthy adults: the ATTICA Study. Am J Clin Nutr 2004;80 (4): 849-54